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THE INFLUENCE OF PREEXISTING IMMUNITY TO ONE OF THE ANTIGENS OF THE ASSOCIATED PLAGUE, TULAREMIA AND BRUCELLOSIS VACCINE ON THE IMMUNIZING PROPERTIES OF ITS OTHER COMPONENTS

Report II

Immunological Reorganization During Complex Vaccination Against Plague, Tularemia and Brucellosis After a Previous Inoculation With Live Tularemia Vaccine

[Following is the translation of an article by V.G. Pilipenko, Stavropolskiy Branch of the "Mikrob" All-Union Scientific-Research Antiplague Institute, published in the Russian-language periodical Zhurnal Mikrobiologii, Epidemiologii i Immunobiologii (Journal of Microbiology, Epidemiology and Immunobiology) No. 11, 1966, pages 43-48. It was submitted on 4 April 1966. Translation performed by Sp/7 Charles T. Ostertag, Jr.]

In studying killed associated vaccines several investigators noted that in a number of cases the presence of immunity in respect to one of the antigens of the complex vaccine led to a weakening of the effect of of its other components, introduced primarily in the composition of the combined preparation (Klimentova, 1949-1950; Barr and Johns, 1953). They explained this phenomenon by the fact that the antigen, in respect to which the organism already possessed an increased reactivity, upon the second application of it in the composition of the complex vaccine has a stronger and more rapid effect (revaccination) and displaced the effect of another component, administered for the first time.

This problem, studied relative to killed associated vaccines, has not found the necessary reflection in investigations devoted to live associated vaccines, though the study on the feasibility of using the latter has been going on in the Soviet Union for no less than 10 years.

In Report I we demonstrated that the presence of immunity to brucellosis in guinea pigs did not lower the effectiveness of inoculations with plague and tularemia antigens, administered to the animals in the composition of the appropriate complex vaccine. The formation of immunity in the pigs to plague and tularemia took place on the same level as it did following inoculations with the complex or corresponding monovaccines on animals which had not been subjected to the preliminary immunization with brucellosis vaccine.

In the present report we will present data on the influence of immunity to tularemia on the effectiveness of inoculations with plague and brucellosis antigens, administered to the animals in the composition of the complex vaccine against plague, tularemia and brucellosis.

The tests were carried out on guinea pigs. For immunizing them we used the live tularemia vaccine from the Odessa Institute of Epidemiology and Microbiology (Series No. 195). The vaccine, diluted in 2 ml of distilled water, was administered in a volume of 0.1 ml (245 million microbes) to the animals on a sheared sector of skin on the abdomen in drops - 2 drops at a distance of 2-3 cm from each other. Using a vaccination quill, 4 parallel scarifications were made through each drop, and then the vaccine was rubbed in with the flat side of the quill.

The course of the local postvaccination reaction was followed in 20 out of the 80 animals inoculated up until its complete attenuation. In 6 of these animals the duration of the reaction equalled 6, and in 14 animals - 8 days. The reaction was expressed in the form of redness and an infiltrate within the limits of the square made by scarifications or somewhat more (an average of 7 x 10 mm).

The indices of immunological reorganization in respect to tularemia were checked in 39 guinea pigs in 60-65 days after inoculation. A positive reaction to tularin was noted in 38 pigs in the form of reddening and an infiltrate with dimensions ranging from 10x11 up to 18x25 mm. At the same time the Burnet test with brucellin was set up on these same pigs. It turned out negative in all the animals. Prior to the tularin test on the pigs blood was taken for setting up the agglutination reaction with the tularemia diagnostic agent. A positive agglutination reaction was obtained with the sera of 34 pigs out of 38 (89%). Of these, the sera of 10 pigs reacted positively in a dilution of 1:10, 13 pigs - 1:20, and 11 pigs - 1:40. The average titer was 1:26. These sera did not react with the brucellosis diagnostic agent.

Thus, the resulting indices of the sero-allergic reorganization testified that the guinea pigs, inoculated with the tularemia vaccine, at the moment of investigation were found in a state of specific immunological reactivity to the primary administration of the tularemia antigen. In this period 40 pigs out of the 80 were additionally inoculated with the complex vaccine against plague, tularemia and brucellosis. For the inoculation we used washings from 2-day agar cultures, cultivated from the dry monovaccines from the same series which were used for the initial immunization of the animals.

Using a glass pipette (0.05 ml) we placed one drop of each of the vaccines on separate sectors of skin on the abdomen at a distance of 2-3 cm from each other. The drop of tularemia vaccine contained 375 million, the drop of plague - 3 billion, and the drop of brucellosis vaccine - 8-9 billion of the corresponding live bacteria. All 3 vaccines were inoculated separately in order to better observe the course of the local reaction to the inoculation of each of them. On the 2nd day after inoculation the average size of the sector with the local reaction (redness, infiltrate) equaled 12x16 mm for the tularemia vaccine, 10x13 mm for plague, and 10x15 mm

for brucellosis. In these tests the average area of the reaction to the tularemia vaccine, which proceeded most likely of all as an allergic type, was greater than during the primary inoculation of this preperation (7x10 mm). In the majority of cases the duration of the reaction to all the vaccines equaled 6-8 days.

Two months after immunization with complex vaccine we checked the indices of the sero-allergic reorganization in the animals in respect to the brucellosis antigen. For this purpose 20 pigs out of 40 received brucellin intracutaneously (based on Burnet)*. In all the animals the brucellin probe turned out positive and was expressed in the form of an infiltrate and redness, encompassing an area from 15x15 up to 25x30 mm, (an average of 20x22 mm). In addition to this the blood sera from 19 pigs were investigated in the agglutination reaction with brucellosis diagnostic agent (based on Wright). In all the pigs a positive reaction was obtained in dilutions from 1:20 to 1:360 (an average of 1:92).

*Brucellin for intracutaneous application, Series No. 16, expiration date 20 July 1965, dose 0.1 ml, administration strictly intracutaneously.

Verification of the resistance of the pigs to 2 infecting doses of a virulent strain of Br. melitensis was carried out after 3 months and in later periods after the inoculation with the complex vaccine. The check showed that there were no essential difference in the number of brucellosis immune animals, subjected to immunization with tularemia vaccine and then with complex, or inoculated only with the complex (Table 1).

In the first two tests the Br. melitensis No. 543 culture used by us possessed a lowered virulence, as a result 4 of the control animals out of 9 were not infected with brucellosis. However, we considered it feasible to cite the data from these tests, since in them in 55% of the control animals a generalized form of brucellosis developed, while among the vaccinated animals less than 10% were infected with brucellosis.

In the 4th and 5th test we used another virulent strain of Br. melitensis - No. 565. In these tests 70-80% of the pigs, immunized with complex vaccine 8 months after inoculation with tularemia vaccine and infected 9 months after inoculation with the complex preparation, turned out to be resistant to 2-4 infecting doses of Br. melitensis No. 565. A generalized form of brucellosis was observed in all the control immunized animals.

Thus, the data obtained testify that the presence of immunity against tularemia in animals did not lead to a lowering in the effectiveness of inoculation with brucellosis artigen, administered to the animals as a component of a complex vaccine.

The influence of immunity to tularemia on the effectiveness of inoculation with the plague antigen, administered to pigs as part of a complex

vaccine, was determined with the help of the allergic pestin test by means of infecting the animals with a highly virulent strain of P. pestis. For checking the allergic reorganization in respect to the plague antigen, just as in the previous tests (Report 1), we used purified pestin (PP - polysaccharide, polypeptide complex) Series No. 3 (V), prepared at the Saratov "Mikrob" Institute. The pestin was administered intracutaneously in a volume of 0.1 ml. Five groups of animals were subjected to the testing. In the 1st group 10 pigs were inoculated with the complex vaccine 2½ months after immunization with tularemia vaccine, the check with pestin was performed 2 months after the inoculation with the complex vaccine; 5 pigs in the 2nd group were inoculated with complex vaccine 8 months after immunization with tularemia monovaccine, the check with pestin was performed 8 months after the inoculation with the complex vaccine; the pigs of the 3rd and 4th groups, inoculated respectively with tularemia and brucellosis monovaccine, served as the control; the 5th group contained nonimmunized pigs.

In all the pigs of the 1st and 2nd groups there was a positive reaction to pestin. It was expressed in the appearance of an infiltrate and hyperemia with a dimension of 18x19 - 15x15 mm in the first and second 24-hour periods. A reaction with a duration up to 3 days was had by all the pigs, then it faded away and only in several pigs was it maintained up to 5-7 days. In all of the pigs in the 3 control groups there was no reaction to pestin.

The presence of immunity to plague was checked in 4 groups of guinea pigs. The animals of the 1st group were immunized with tularemia vaccine and once more with complex vaccine, the animals of the 2nd group - only with complex. The interval between inoculations was 2½ months. The animals of these two groups were subjected to infection 3½ months after the inoculation with complex vaccine. The pigs of the 3rd and 4th groups were subjected to an analogous influence with the only difference that the interval between the inoculations with tularemia and complex vaccines was 8 months, and between the inoculations with complex preparation and infection - 11½ months. The pigs of the first two groups were infected with a culture of P. pestis No. 261; one unconditionally lethal dose of this for guinea pigs equaled 50 microbial cells; for infecting the animals of the 3rd and 4th groups we used the standard strain of P. pestis No. 461, an unconditionally lethal dose of which equaled 10 microbial cells for guinea pigs. Each pig was given 200 unconditionally lethal doses of the plague causative agent.

In tests 1 and 3 all the pigs turned out to be resistant to infection with 200 Dcl of the plague causative agent both after 3½ months and after 11½ months following inoculation with the complex vaccine (Table 2). The pigs receiving only the complex vaccine turned out to be less protected, particularly the pigs of the 4th group, which were infected with plague in later periods after the inoculation (after 11½ months). We obtained analogous data in animals, which prior to immunization with complex preparation in place of tularemia were inoculated with brucellosis vaccine (Report I). These facts, from our point of view, merit attention and further checking,

since they permit the assumption that the condition of postvaccinal immunity against tularemia (and equally brucellosis) in animals following subsequent immunization with complex preparation causes a clear effect of revaccination in respect to the plague antigen.

In this manner the data from checking the immunity to plague, and also the indices of the allergic reorganization in respect to the plague antigen, make it possible to draw the conclusion that the presence of immunity to tularemia did not lead to a lowering in the effectiveness of inoculation with the plague antigen. On the other hand, stemming from the data obtained, it can be assumed that immunity to tularemia increases the immunizing properties of plague antigen, administered to an animal as part of a complex vaccine.

In pigs, immunized with tularemia vaccine, and then with complex we also checked the shifts in immunological indices in respect to the tularemia antigen. For this purpose 20 pigs were given the tularin test by means of the intracutaneous administration of 0.1 ml of ordinary tularin. The test was positive in all the pigs. The dimensions of the sector in the form of redness and an infiltrate equaled 10x10 - 20x29 mm (an average of 16x17 mm). The sera of all 20 pigs were tested in the agglutination reaction with the tularemia diagnostic agent. With the sera of 14 pigs (70%) a positive reaction was obtained in dilutions of from 1:10 to 1:80 (average titer 1:20).

Checking the resistance of the pigs to tularemia was performed by means of infecting the animals with 1000 unconditionally lethal doses of a virulent culture of F. tularensis No. 719. For guinea pigs, one lethal dose of this equaled 1 microbial cell (based on the standard of the State Control Institute). Three groups of immunized pigs were subjected to infection. The animals of two groups were inoculated with tularemia vaccine and additionally with complex vaccine, only with a different interval between inoculations. The animals of one group were inoculated only with the complex vaccine. In the 1st group the loss of animals from tularemia was somewhat higher (Table 3), though it seemed that it should have contained more resistant specimens, since the pigs in this group initially were inoculated with tularemia vaccine, and after 2 months were subjected to revaccination with the tularemia antigen, administered as part of the complex vaccine. Besides this, as was already indicated above, in this group the number of pigs, the sera of which contained tularemia agglutinins, was less than among those inoculated only with the tularemia vaccine. It can be proposed that the use of complex vaccine to inoculate animals, which are found at the peak of the response reaction to the primary administration of tularemia vaccine, instead of strengthening immunogenesis in respect to the tularemia antigen caused a certain inhibition of it. However, taking into consideration the small difference in the number of animals which died in the 1st and 2nd groups (all told 2 pigs), and the fact that a small number of animals with tularemia agglutinins was in the 2nd group, which was inoculated only with the complex vaccine, it is more advisable to restrain yet from such a conclusion and to continue the investigation.

Conclusions

1. The presence of immunity to tularemia in guinea pigs did not lower the effectiveness of inoculations with brucellosis and plague antigens, administered to the animals as part of a complex trivaccination.

2. The formation in guinea pigs of an immunity to brucellosis and plague took place on the same level (for plague even higher) as after the inoculation with complex or the appropriate monovaccines of animals which were not subjected to preliminary immunization with tularemia vaccine.

Table 1

Checking immunity to brucellosis in guinea pigs, inoculated with tularemia and additionally complex vaccine

No. of test	Vaccine	Interval between inoculations (months)		Number of infected animals	Outcome of infection			Number of immune animals	
		mono and complex vaccines	complex vaccine and infection		generalized infection	regional infection	not infected	abs.	%
1	Tularemia + complex	2½	3	12	1	-	11	11	91
	Complex	-	3	12	1	1	10	11	91
Control				9	5	-	4	-	-
2	Tularemia + complex	8	9	10	3	3	4	7	70
	Complex	-	9	10	4	1	5	6	60
Control				10	10	-	-	-	-

Table 2

Checking immunity to plague in guinea pigs, inoculated preliminarily with tularemia vaccine and once more with complex trivaccine.

No. of test	Vaccine	Interval between inoculations (months)		Number of infected animals	Outcome of infection		
		mono and complex vaccines	complex vaccine and infection		died from plague	survived	% immune
1	Tularemia + complex	2½	3½	12	0	12	100
2	Complex	-	3½	12	2	10	83
Control		-	-	12	12	-	--
3	Tularemia + complex	8	11½	11	-	11	100
4	Complex		11½	12	8	4	33
Control		-	-	10	10	-	--

Table 3

Checking immunity to tularemia in guinea pigs, preliminarily inoculated with tularemia vaccine and additionally with complex trivaccine.

No. of test	Vaccine	Interval between inoculations (months)		Number of infected animals	Outcome of infection		
		mono and complex vaccines	complex vaccine and infection		died from tularemia	survived	% immune
1	Tularemia + complex	2½	2	12	3	9	75
2	Complex	-	2	12	1	11	91
Control		-	-	10	10	-	--
3	Tularemia + complex	8	2	10	1	9	90
Control		-	-	10	10	-	--